SPE Application Note for
Extraction of Catecholamines from Urine

This method details the use of a non-polar retention mechanism for the extraction of catecholamines (noradrenaline, adrenaline, dopamine) from urine. The polar analytes form a non-polar complex with phenylboronic acid and are retained on the non-polar MFC18 column. Typical recoveries are >85%.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: ISOLUTE MFC18 100 mg/10 mL, Part number 240-0010-G. For automation compatibility, ISOLUTE MFC18 100 mg/1 mL, Part number 240-0010-A offers the same sorbent bed dimensions.

Pre-treatment: In a suitable glass container, add:-
1. 1 ml urine sample (or calibrator, or QC)
2. 100 uL internal Standard (1.2 ug/mL in 0.01 mol/L HCl
3. 2 mL of buffer containing complexing agent Vortex well to mix. The pH should be 7.5-9.5 - check pH of all the samples and adjust with ammonia if required.

Solvation: Solvate the column with methanol (2 ml) at a flow rate of 0.5-1 mL/min
Do not allow the column to dry out.

Equilibration: Equilibrate with ammonium chloride wash buffer (0.2 mol/L, 2 mL) at a flow rate of 0.5-1 mL/min, then switch off the vacuum.
Do not allow the column to dry out.

Sample application: Take a 1.5 mL aliquot of the sample and apply to the column at a flow rate of ~0.5 mL/min (vacuum -2 to -5" Hg). The total flow time should be 2-3 min.
Do not allow the column to dry out.

Interference elution: Set the pressure to <-5"Hg.
1. Wash with ammonium chloride wash buffer (0.2 mol/L, 2 mL)
2. Wash with 20% methanol wash buffer (2 mL)
3. Dry for 30 sec on full vacuum (-20" Hg)

Analyte elution: To elute analytes, apply first volume of elution solvent to extraction column. Soak for two minutes. Add second volume of elution solvent to extraction column and collect.

Elute with acetic acid (1 mol/L, 1.5 mL) into glass tubes. The pressure should be ~5" Hg.

Structure	Not shown.
Structural considerations	Catecholamines are polar, with cis-hydroxy groups that interact with phenylboronic acid (complexing agent) to produce a less polar complex that is retained on a C18 column by non-polar interactions. Elution is achieved by breaking the interaction with acidic conditions.
Matrix considerations	See general comments for possible interferences.
Analytical method	HPLC with ESA detection. Column: C18, 5um, 4.6 x 250mm (Nucleosil or Apex) Mobile phase: 100 mg SDS and 50 mg EDTA in 140 ml phosphate buffer (50 mol/L). Add 40 mL acetonitrile and 20 ml methanol. Adjust pH to 2.9 using phosphoric acid. Filter before use. Flow rate: 1.5 mL/min Injection volume: 40 ul Detection: Electrochemical detector (ESA); model 5100 A Gain 10x10; det. 1:0.1V, det. 2:0.35V
Reagents	1. 2.0 mol/L ammonium chloride - ammonium hydroxide buffer: Dissolve 107g NH4Cl in 1 litre of deionized water and adjust pH to 8.5 with NH4OH. Store at 4 degC. 2. Buffer containing complexing agent: To 200 mL of above buffer, add 400 mg diphenylboronic acid ethanolamine ester (obtained from Aldrich, p/n 12630-5) and 1g disodium EDTA. Diphenylboronic acid does not dissolve easily and may require to mixing slowly overnight to dissolve completely. Adjust pH to 8.5 with ammonia. Store at 4degC and check pH before use. 3. Wash buffer (0.2 mol/L NH4Cl-NH4OH): Add 50 ml of 2 mol/L NH4Cl-NH4OH buffer to a 500 mL volumetric flask and make up to 500 mL with distilled deionized water. Add 250 mg EDTA and adjust pH to 8.5 with ammonia. Store at 4 C and check pH before use. 4. Methanol wash buffer (20% MeOH in wash buffer): Add 100 mL MeOH to 400 mL of 0.2 mol/L NH4Cl-NH4OH buffer and adjust pH to 8.5 with ammonia. Store at 4 C and check pH before use. 5. 1.0 mol/L acetic acid: Add 12 mL of glacial acetic acid to a 200

mL volumetric flask and make up to 200 mL with distilled deionized water.

General comments

a) N.B. Urine samples should be acidified on collection to pH 1-3
b) Do not allow the column to dry out unless otherwize stated. This will prevent irreversible analyte adsorption.
c) Set vacuum to <-5"Hg (-2-3"Hg)
d) Sample loading and elution flow rates should not exceed 0.5 mL/min
e) Once the complexing agent has been added to the sample, the complex is stable for at least 2 hrs at room temperature.
f) Complexing agent - forms a non-polar complex with the analyte (due to cis hydroxy functional groups), which is retained on the non-polar column. The elution is achieved by breaking the interaction with acid conditions.
g) The methanol concentration in the methanol wash buffer should not exceed 20%.
h) MFC18 columns must be used for catecholamine extraction. Endcapped C18 sorbents give poor recoveries.
i) Structurally similar drug metabolites may interfere with the analytes of interest. Metabolites of paracetamol and labetalol interfere with measurement of adrenaline. Urine from patients on methyl dopa produces a Iarge peak before noradrenaline (potential to interfere). Urine from patients on atenalol or capropril show no interference. Reference: With thanks to Dinesh Talwar, Dept. of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, UK. 2001

ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

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